

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
27 February 2003 (27.02.2003)

PCT

(10) International Publication Number
WO 03/016883 A1

- (51) International Patent Classification⁷: G01N 21/76 (74) Agents: DERRICK, Wesley, B. et al.; Needle & Rosenberg, P.C., 127 Peachtree Street, N.E., Atlanta, GA 30308-1811 (US).
- (21) International Application Number: PCT/US02/26019
- (22) International Filing Date: 16 August 2002 (16.08.2002) (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/313,196 16 August 2001 (16.08.2001) US
- (63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 60/313,196 (CIP)
Filed on 16 August 2001 (16.08.2001)
- (71) Applicant (*for all designated States except US*): ANALIZA, INC. [US/US]; 408 Glen Park Drive, Bay Village, OH 44140 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (*for US only*): CHAIT, Aaron [US/US]; 408 Glen Park Drive, Bay Village, OH 44140 (US). ZASLAVSKY, Boris, Y. [US/US]; 34200 Country View Lane, Solon, OH 44139 (US).
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/016883 A1

(54) Title: A METHOD OF MEASURING SOLUBILITY

(57) Abstract: A method of determining the solubility of a compound in a selected solvent is provided that does not require determination of, or use of, standards having known concentrations of the compound. In one aspect, the method can include preparation of a mixture where not all of a compound is dissolved in the provided solvent, separating undissolved compound from the solvent, and direct determination of the amount of the compound dissolved in the solvent. Methods adapted for use include those where a multiplicity of compounds or solvents are tested in parallel. Devices adapted for these methods are also provided by the present disclosure.

5

METHOD FOR MEASURING SOLUBILITY

This application claims priority to U.S. Provisional Application Serial No. 60/313,196, filed August 16, 2001, which application is herein incorporated by reference in its entirety.

10

FIELD OF THE INVENTION

In general, the disclosed invention is directed to methods allowing determination or estimates of the miscibility, solubility or other similar properties of compounds in selected or chosen solvents. More particularly, the invention relates in part to methods for facilitating measurements of the solubility of organic, inorganic and organo-metallic compounds, particularly of compounds related to pharmaceutical and agrochemical research and development.

BACKGROUND

20

In technical fields relating to chemical formulation of compounds, such as, but not limited to the fields of pharmaceutical and agrochemical research and development, it is almost always necessary to evaluate the general suitability of a newly developed drug candidate prior to launching into full development. Such an evaluation of the general suitability or, in the field of pharmaceutical development, drug-ability of such chemical compounds typically includes solubility studies of the compound in various

5 solvents as well as solubility profiles at various pH values.
However, carrying out such studies for a great many compounds can
be problematic and resource-intensive. At the earlier stages of
the drug discovery process, in particular, the solubility
measurements are generally performed for a large variety of
10 compounds. Furthermore, many of these compounds are only available
in limited quantities, either due to difficulties in manufacturing
larger quantities, handling all but the smallest sized samples, or
simply because the cost of producing or handling larger quantities
of the compounds is not feasible.

15 However, simply bypassing the solubility studies is also not
a viable option for product development as selection of an
otherwise suitable candidate compound that does not have a suitable
solubility profile can cause significant problems. Indeed,
insoluble or poorly soluble compounds often prove difficult to
20 develop into drugs. Even with significant motivation, the
development of low-solubility drugs is more time-consuming and
expensive than for a compound with otherwise more suitable
properties.

Traditionally, "equilibrium" solubility has been determined
25 by agitating or shaking the compound with the solvent of choice for
at least 24 hours or until no more of the compound will dissolve,
then filtering, and determining the concentration of dissolved
compound by a suitable analytical assay. These analytical assays
had to be calibrated, a process which includes preparation of at
30 least several solutions of the known varied concentrations of the

5 compound (standard solutions), and establishing a quantitative relationship between a measurable analytical signal and the compound concentration. This approach is inappropriate in a modern drug discovery setting. The throughput, number of unknown samples that can be determined in a given amount of time or using a given
10 quantity of resources, such as machines, personnel, samples, and the like, is not high enough to meet the required demand to analyze a great number of potential lead compounds. For example, determination of the mass of samples and/or standards presents too restrictive a checkpoint in the process for maintaining the high
15 throughput desired as the process of weighing hundreds (or thousands) of solid samples in submilligram quantities.

Therefore, to alleviate the perceived hindrances to high throughput analysis of compounds, those of skill in the art have sought to develop improved methods for determining solubility of
20 compounds. One of these methods is based on measuring turbidity of an aqueous media after adding a fixed amount of solution of a compound in dimethyl sulfoxide (DMSO) by using laser nephelometry (Bevan & Lloyd, "A high-throughput screening method for the determination of aqueous drug solubility using laser nephelometry
25 in microtiter plates," *Anal.Chem.* 72, 1781-1787 (2000)). However, this method is limited in that it does not allow one to measure solubility of compounds in pure aqueous media without DMSO. Another method suggested in the literature is based on measuring the vapor pressure depression for a solution of the compound at
30 saturation (Parikh et al., "Rapid solubility determination using vapor-phase osmometry," *J. Biomol. Screen.* 4, 315-318 (1999)).

5 However, this method is limited to use for measuring the solubility
of nonionic compounds with rather good solubility in pure water.
Furthermore, it cannot be used for poorly soluble compounds, and
its use for solutions of ionic compounds in buffer or salt
solutions is considered questionable (Parikh et al., "Rapid
10 solubility determination using vapor-phase osmometry," *J. Biomol.*
Screen. 4, 315-318 (1999)).

Additional methods, such as those available from pIon Inc.,
are based on producing experimental samples by mixing DMSO solution
of a compound of interest with a given aqueous solvent, incubation
15 of the mixture for a fixed period of time, removing the precipitant
formed by filtration, and assaying the compound concentration by
measuring the optical absorbance of the filtrate at the maximum
wavelength specific for the compound. However, differences between
these assays and those to determine the concentration of compounds
20 to use or to determine the optical absorbance values to use in
these additional methods give rise to difficulties. For example, a
concentration assay (similar to those used to determine solubility,
but without incubation and filtration) is performed in a separately
prepared mixture of the same DMSO solution of the compound with the
25 same aqueous solvent but using a higher ratio of DMSO to aqueous
solvent in the mixture. Under suitable conditions, the higher
DMSO/aqueous solvent ratio is such that the compound is not
precipitated out of solution. Correspondingly, the measurement of
absorbance is taken to indicate the absorbance for a given quantity
30 of the compound. This measurement when all the compound is
solubilized, or rather, sets of these measurements are used as

5 reference points to generate a relationship between the measured
absorbance value and the quantity of compound. In determine the
relationship, essentially a standard curve as is known in the art,
the linearity of the optical absorbance vs. concentration over the
used DMSO/aqueous solvent ratio range is assumed. Comparison of
10 the standard curve and the measured value determined from the
experimental sample, following incubation and filtration, is used
to calculate/determine the concentration of the compound in the
experimental sample.

In general, procedures like those available prior have
15 additional disadvantages which limit their practical application
under some circumstances. In particular, the technique as outlined
above requires that it be possible to determine the initial
concentration of a compound under study in DMSO or in a DMSO-
containing solvent. Further, this method is limited to compounds
20 with chromophoric groups, such that they can be detected by
absorbance measurements. Procedures that do not require that the
compounds to be amenable to their initial concentration being
determined in DMSO or a similar solvent or that do not require that
the compounds have easily detectable or commonly used chromophoric
25 groups would be a significant advancement of the technology to test
the solubility of compounds.

The present invention provides new methods for determining
the solubility of compounds of interest in solvents of interest.
In specific embodiments, this includes methods that allow
30 measurement of the solubility of organic, inorganic and organo-

5 metallic compounds, particularly of compounds related to
pharmaceutical research and development, in aqueous media with or
without organic solvent. Further, the provided method is not
necessarily limited by the ionization state of the compound or by
the presence of inorganic salts and/or buffer salts in the media.
10 Further, the provided methods are adaptable for high-throughput
automated measurements. These and other objectives of the
invention will become apparent in view of the detailed description
below.

SUMMARY OF THE INVENTION

15 In accordance with the purpose(s) of this invention,
as embodied and broadly described herein, this invention,
in one aspect, relates to a process of measuring the
solubility of a compound in a selected solvent, comprising
the steps of: preparing a mixture of a quantity of the
20 compound with a volume of the selected solvent; incubating
the mixture of compound and solvent, whereby compound can
dissolve in the solvent to form a solution of the
compound; removing undissolved compound from the mixture,
thereby providing a quantity of the solution of the
25 compound; determining the amount of a selected constituent
of the solution of the compound resulting from removing
undissolved compound from the mixture, wherein the
determination does not include a comparison of physical
properties of one or more solutions containing known
30 concentrations of the compound and physical properties of

5 the solution of the compound; and calculating the solubility of the compound in the selected solvent by determining the amount of the compound present in a determined quantity of the solution of the compound.

10 In a second aspect, the present invention relates to a method for determining the solubility of chemical compounds, including organic, inorganic and organo-metallic compounds. The method can include: preparing a mixture of unknown excess amount of compound sample with a fixed volume of a solvent of choice; maintaining a sample
15 of the aforementioned mixture until thermodynamically equilibrated phase separation occurs; withdrawing aliquots of the saturated solution from the thermodynamically equilibrated mixture; analyzing the concentration of the compound in the solution in aliquots withdrawn from the
20 saturated solution by measuring the overall content of a given chemical element in a fixed volume of the solution; and converting the overall content of a given chemical element in the solution into the concentration of the tested compound from the molar content of the chemical
25 element in the compound, and the detector-specific universal quantitative relationship between the element content and quantity of the element-containing compound in a fixed solution volume.

30 In a third aspect, the present invention relates to a method for determining the solubility of one or more

5 compounds in one or more selected solvents. The method can include preparing two or more mixtures of compound and solvent and determining the solubility of each according to any method according to the first and second aspects of the invention.

10 In a fourth aspect, the present invention relates to an apparatus for determining the solubility of a compound in a selected solvent. The apparatus can include: a mixing device that combines a quantity of a compound with a volume of a selected solvent, thereby forming a mixture
15 in a container; an incubating device that maintains the mixture at determined conditions for, optionally, a determined period of time; a separating device that removes undissolved compound from the mixture, thereby providing a quantity of a solution of the compound; and a
20 detector that detects the amount of a selected constituent in the solution of the compound. The apparatus can be adapted to any method according to the first, second or third aspects of the invention.

In certain embodiments, the present invention is
25 directed to a process of measuring solubility of organic, inorganic and organo-metallic compounds, particularly of compounds related to pharmaceutical, cosmetic, and agrochemical research and development, in aqueous media without organic solvent not limited by ionization state of
30 the compound or the presence of inorganic salts and/or buffer salts in the media and adaptable for a high-

5 throughput automated measurements. In certain
embodiments, the method used can be based on dispersing an
unknown-weight quantity of a compound in a solvent of
choice by shaking, sonication, or other means, removing
the non-dissolved compound by filtration, centrifugation,
10 or other means, and measuring total amount of a
constituent present in the dissolved compound molecule.
Constituents detected and measured can include, but are
not limited to, particular moieties, groups, or chemical
elements, such as nitrogen, carbon, or sulfur.

15

BRIEF DESCRIPTION OF THE DRAWINGS

The following is a brief description of drawings,
which form a portion of the specification, and are
presented for the purpose of illustrating selected aspects
of the invention. The drawings are incorporated in the
20 specification and together with the description serve to
explain the principles of certain aspects of the
invention.

FIGURE 1 illustrates the relationship between the
concentration of caffeine in solution measured by the
25 total nitrogen content of the solution and the nominal
concentration values for the prepared caffeine solutions.

FIGURE 2 illustrates the relationship between the
solubility values obtained according to the present
invention and the literature solubility data.

5 FIGURE 3 illustrates the relationship between the
solubility data obtained for different compounds according
to the present invention as measured by the total nitrogen
content of the saturated solution in water and as measured
by the total carbon content of the saturated solution in
10 water.

FIGURE 4 illustrates the relationship between the
solubility data obtained for different compounds according
to the present invention as measured by the total nitrogen
content of the saturated solution in buffer at pH 7.4 and
15 as measured by the total carbon content of the saturated
solution in buffer at pH 7.4.

FIGURE 5 illustrates the relationship between the
solubility data in the presence of 2% DMSO obtained for
different compounds according to the present invention as
20 measured by the total nitrogen content of the saturated
solution and the solubility of the same compounds without
DMSO.

FIGURE 6 illustrates the relationship between the
solubility data in the presence of 2% methanol obtained
25 for different compounds according to the present invention
as measured by the total nitrogen content of the saturated
solution and the solubility of the same compounds without
DMSO.

5

DETAILED DESCRIPTION OF THE INVENTION

Before the present compounds, compositions, articles, devices, and/or methods are disclosed and described, it is to be understood that this invention is not limited to specific methods, specific solutions, or to particular
10 devices, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. Throughout the specification and claims, reference will be made to a number of terms
15 which shall be defined to have the following meanings:

As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "solvent" includes
20 mixtures of solvents, and the like.

Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes- from the one particular value and/or to the
25 other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant

5 both in relation to the other endpoint, and independently
of the other endpoint.

"Optional" or "optionally" means that the
subsequently described event or circumstance may or may
not occur, and that the description includes instances
10 where said event or circumstance occurs and instances
where it does not.

In certain contexts, solubility of a compound in a
solvent media is defined as the compound concentration in
the solvent at saturation. In other contexts, solubility
15 of a compound in a selected solvent can be defined as the
compound concentration in solution under specified
conditions wherein the quantity of the compound in contact
with the solution is not a significantly limiting factor
(for instance, where a solid compound is added to a
20 solvent and solid compound remains undissolved after a
specified period of time). In another context, solubility
of a compound can mean determination that under specified
conditions, or that after following a specified procedure,
the compound remains in solution at the specified
25 concentration. If solubility of the compound is used in
this context, the determined solubility of the compound
relates to a limit of the compound's solubility, namely,
that the compound must at least be soluble at the
determined concentration, although it can be possible to
30 have higher concentrations of the compound in solution.
Use of such determinations can provide adequate

5 characterization of a compound's characteristics in regard
to its ability to dissolve in a particular solvent or to
remain in solution with a particular solvent to be used as
guidelines in developing protocols or processes as will be
recognized by those of skill in the art.

10 The measurement of the solubility of a compound
includes a number of steps, including the preparation of a
saturated solution of the compound and measuring the
quantity of the compound present in the solution. In the
use of standard methods; the preparation of a saturated
15 solution requires weighing out a given quantity of dry
compound into a vessel and adding a fixed amount or volume
of a solvent, dispersion of the compound in the solvent,
removing the non-dissolved fraction of the compound, and
measuring the quantity of the dissolved compound in the
20 remaining solution.

As normally practiced, preparation of the saturated
solution (particularly, weighing out the compound and
adding a fixed amount of solvent) and measuring the
quantity of dissolved compound present in solution
25 remaining after removing nondissolved compound require
most of the time and manpower. In part, this is due to
the fact that the dispersion of compound can be performed
in parallel for a multiple set of samples by shaking,
sonication, mixing, and the like and that removing
30 nondissolved compound can be performed in parallel for a

5 multiple set of samples by filtration, centrifugation, and the like.

When assaying the solubility of even a small number of compounds, standard methods can often require weighing of hundreds of solid samples, generally in sub-milligram
10 quantities. Further, the step of measuring the quantity of dissolved compound in the remaining solution following removal of nondissolved compound generally requires the use of an analytical assay that is commonly compound-specific. Whenever the assay is compound specific,
15 separate assay development for each compound to be studied can be required. This requirement for extensive assay development can cause excessive expenditure of resources.

In one aspect, the present invention relates to the method of measuring the solubility of a compound in a
20 selected solvent that includes the steps of preparing a saturated solution of the compound, wherein the saturated solution is prepared by combining a quantity of compound with a volume of the selected solvent, wherein the volume of selected solvent is not adequate to fully solubilize
25 the compound and measuring the amount of compound solubilized in a given quantity of the resulting solution. The method can include the steps of: combining a quantity of the compound with a volume of solvent; incubating a volume of the combined compound and solvent for a period
30 of time, optionally with action to disperse the compound in the volume; removing undissolved compound from at least

5 a portion of the resulting solution of dissolved compound in the solvent; and determining the quantity of compound dissolved in a quantity of solvent.

The compound whose solubility is tested can be in solid, liquid or gaseous form, or a combination of these
10 forms. For example, a slurry of a compound in another solvent can be provided to a solvent for which the solubility of the compound is to be tested.

The amounts of compound provided can vary in accordance with the necessary requirements imposed by the
15 materials tested. For example, when testing compounds of very high solubility in a given solvent, the quantity of material provided relative to the volume of solvent provided will necessarily be greater than is needed when compounds of very low solubility in a given solvent are
20 tested. Thus, quantities of compounds used can be greater than 1, 2, 5, 10, 30, 100, 250, 500 or 1000 pico-, nano-, micro- or milli-grams or less than 1, 2, 5, 10, 30, 100, 250, 500 or 1000 pico-, nano-, micro- or milli-grams. Thus, volumes of solvent used can be greater than 1, 2, 5,
25 10, 30, 100, 250, 500 or 1000 nano-, micro- or milli-liters or less than 1, 2, 5, 10, 30, 100, 250, 500 or 1000 nano-, micro- or milli-liters. As recognized by those of skill in the art, solvents can be a combination of one or more compounds (i.e., a solvent can be a solution).

5 The process or step of dissolving / suspending sample
can be one step or it can be more than one step. For
example, the sample can be suspended in a smaller volume
of a solvent and then provided to a larger volume of a
second solvent (for example, as a slurry or as an agitated
10 suspension). Alternatively, the compound can be dissolved
in a first solvent and then provided to a second solvent
in which the compound may not be fully soluble. For
example, a quantity of a compound can be dissolved in a
small volume of organic solvent and then added to a volume
15 of an aqueous solvent whereupon at least a portion of the
compound comes out of solution. For example, if the
organic solvent remains in the solution during the
subsequent measurement, the solubility can be taken to
mean kinetic or apparent solubility which may or may not
20 correspond to "equilibrium" solubility. These
measurements can be used for high throughput comparison of
compound to order or to rank their apparent solubility.
In some embodiments wherein the compound is suspended or
dissolved and then added to a further solvent, the first
25 solvent can be a volatile solvent that can be removed from a
solution formed by addition of any further solvent. In
such embodiments, the removal of the first volatile
solvent can cause at least a portion of the compound
suspended or dissolved in the first solvent to come out of
30 solution. Measurement of the amount of compound remaining
in the solvent remaining at the end of incubation can

5 thereby allow measurement of the solubility of the
compound in the remaining solvent.

Dispersion of compound throughout at least a portion
of a volume of solvent can be facilitated by stirring,
shaking, sonication, as well as by other forms of
10 mechanical agitation as are known to those of skill in the
art. Such efforts to effect or increase dispersion can be
continuous throughout the desired incubation or can be
intermittent.

Incubation of mixtures of compound and solvent can be
15 conducted for lengths of time that are appropriate for the
particular compounds and solvents used, as well as other
conditions which impact solubility, such as, but not
limited to temperature and pressure. Incubation can be at
temperatures greater than -25, -15, -10, -5, -2, 0, 2, 5,
20 10, 15, 20, 25, 30, 35, 40, 50, 60, 80 or 100°C or less
than -25, -15, -10, -5, -2, 0, 2, 5, 10, 15, 20, 25, 30,
35, 40, 40, 60, 80, or 100°C. Incubation can be at
pressures greater than 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7,
0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.8, 2.0, 3,
25 or 5 atmospheres or at pressures less than 0.1, 0.2, 0.3,
0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4,
1.5, 1.6, 1.8, 2.0, 3, or 5 atmospheres.

Following incubation, removal of undissolved compound
from the volume of solvent and dissolved compound can be
30 removed. Methods that can be used to remove undissolved

5 compound include those relying on mechanical entrapment, such as filtration, and those relying on differences in the properties of the dissolved compound/solution and undissolved compound, such as centrifugation or sedimentation.

10 Thus, according to certain aspects of the invention, the procedure of providing a quantity of compound and a volume of solvent for preparing a saturated solution is simplified and does not require the most time- and labor-consuming part, namely, the process of weighing samples.

15 Instead, the procedure used for distributing a quantity of compound need only include transfer of a quantity of the compound that into a vessel that is greater than the amount of compound that can be brought into solution by the provided volume of solvent. Consequently, the amount

20 of the sample need only be defined loosely as exceeding a specified value as can be determined by one of skill in the art. A trial and error process can be used to determine that the amount of compound used is adequate or that the volume of solvent is not too great by determining

25 that a quantity of compound remains undissolved following incubation to dissolve the compound. In certain embodiments, the present invention allows use of small quantities of a sample, such as one of approximately 0.01, 0.02, 0.05, 0.1, 0.2, 0.3, 0.5, or 1.0 mg, or as one that

30 consists of but a visible quantity of a dry sample on a spatula. The procedure to dispense small quantities of compound can be automated, for example, as dry powder

5 dispensing in a given amount of time under fixed spatula vibration settings and the like.

Detection of the presence of dissolved compound in a solution is also provided by the present invention. The method of detection utilized can be a method that is
10 easily automated. According to certain aspects of the present invention, the detection procedure can consist of measuring total content of a chemical element present in a portion of solvent containing the compound that is present in the molecule of the compound (such as carbon, nitrogen,
15 sulfur, etc.). For example, detection of the amount of carbon present in an aqueous solution of sugar can be used to determine the concentration of sugar present in a volume of sugar. In specific embodiments, the determination will include measurement of the amount of an
20 element in a fixed volume of a compound solution. The measurement of the selected element can be performed with a universal equimolar chemiluminescent nitrogen detector, equimolar chemiluminescent sulfur detector, or total organic carbon detector. In specific embodiments, the
25 element detected can be an element present in the compound that is not present in the solvent alone. For example, the detection of carbon in the use of the method to determine the amount of sugar present in an aqueous solution of sugar. In other specific embodiments, the
30 element detected can be an element present in both the element and the solvent and the determination of the amount of the element present in a specific volume of

5 solution can be used to determine the quantity of the
compound dissolved in the solution, thereby allowing
determination of the solubility of the compound.
Alternatively, in other embodiments, the measurement of
the amounts of more than one element can be measured,
10 wherein the ratio of the elements indicates the amount of
compound dissolved in the solution.

Alternatively, in other embodiments, the amount of an
element present in the compound and the amount of a second
element present in the solvent can be used to determine
15 the relative amount of compound present in a given aliquot
relative to the amount of solvent. In these embodiments
of the invention, the measurement of the volume of
solution measured need not be measured in any manner
independent of determining the amount of elements present
20 in the measured sample.

Detection, measurement or determining the amount of
selected constituent present in a solution of the compound
can include comparison with values determined from
solutions having a known concentration. Alternatively,
25 the detection, measurement or determining the amount of a
selected constituent of a solution of a compound can
exclude comparison of physical properties of solutions
containing known concentrations of the compound and
physical properties of the solution of the compound,
30 namely, the method can be conducted without the use of
standards composed of known amounts or known

5 concentrations of the compound whose concentration is
being determined.

In other embodiments of the invention, an apparatus
which includes; a mixing device that can combine a
quantity of compound with a volume of solvent to form a
10 mixture in a container; an incubating device that can
maintain the mixture at determined conditions for,
optionally, a determined time; a separating device that
can remove undissolved compound from the mixture to
provide a quantity of the solution of the compound; and a
15 detector that can detect the amount of a selected
constituent in the solution of the compound. Other
particular embodiments of devices adapted to conducting
the methods of the invention are those that are recognized
by those of skill in the art as being capable of
20 conducting the presently disclosed methods. These
particular embodiments of devices include those adapted
for automated handling and treatment of samples according
the methods of the present invention.

Experimental

25 The following examples are put forth so as to provide
those of ordinary skill in the art with a complete
disclosure and description of how the compounds,
compositions, articles, devices and/or methods claimed
herein are made and evaluated, and are intended to be
30 purely exemplary of the invention and are not intended to

5 limit the scope of what the inventors regard as their invention.

Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for.

10 Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

Example 1. Measuring concentration of solutions of caffeine of varied concentrations by measuring total

15 nitrogen content in a fixed volume of the caffeine solution.

Caffeine was purchased from Sigma Chemical Company (St. Louis, MO, USA) and used without further purification. Solutions of caffeine in water were

20 prepared at the nominal concentrations from 0.9 up to 83.37 mg/ml. Five of these solutions had undissolved residue and were filtered through 0.45 μ filter. These solutions were injected in triplicate into chemiluminescent nitrogen detector (model 8060, Antek

25 Instruments) equipped with an analytical loop of 5 μ l volume. The results obtained are presented in Figure 1.

The concentrations for the examined caffeine solutions are plotted in Figure 1 versus the nominal concentration values for the prepared solutions. The data

30 given in Fig. 1 indicate clearly that the absolute

5 concentrations determined by measuring total nitrogen
content in a fixed volume of solution provides the right
values for concentrations of the compound.

Example 2. Measuring solubility of different
compounds by the procedure according to the present
10 invention.

Allopurinol, bendroflumethiazide, butamben,
clofazimine, hydroflumethiazide, nifedipine,
nitroflurantoin, nitroflurazone, perphenazine, phenacetin,
tolazamide, and sulfanilamide were purchased from Sigma
15 Chemical Company (St. Louis, MO, USA) and used without
further purification. About 10 mg quantities of each of
these compounds were mixed with 0.5 ml of water, and
shaken for 24 hrs. at room temperature. Undissolved
residue in each solution was removed by filtration through
20 0.45 μ filter, and the solutions were injected in
triplicate into chemiluminescent nitrogen detector (model
8060, Antek Instruments) equipped with an analytical loop
of 5 μ l volume.

The measured nitrogen content for each compound
25 solution was transformed into concentration units, and the
resulting experimental solubility values are plotted in
Figure 2 versus corresponding literature solubility data
for the compounds examined. The plot presented in Fig. 2
may be described by a linear relationship as:

5
$$S_{\text{exp}} = 0.036(\pm 0.018) + 1.005(\pm 0.008) * S_{\text{lit}},$$

$$N = 12; r^2 = 0.9993; s = 0.0596,$$

where S_{exp} is the compound solubility measured experimentally by the procedure described; S_{lit} - solubility of the compound reported in the literature; N
10 is the number of compounds; r - correlation coefficient, s - standard error of estimate.

The data given in Fig. 2 indicate clearly that there is a good correlation between the solubility values obtained by the procedure according the present invention
15 and the literature data for different compound with solubility over the range from 1 $\mu\text{g/ml}$ up to ca.10 mg/ml .

The results illustrated by the above examples demonstrate that the procedures according to the present invention facilitate high-throughput measurements of
20 solubility for a wide variety of compounds.

Example 3. Measuring solubility of several different compounds in water by the procedure according to the present invention using two different assay protocols.

Butamben, bendroflumethiazide, and phenylbutazone
25 were purchased from Sigma Chemical Company (St. Louis, MO, USA) and used without further purification. About 20 mg quantities of each of these compounds were mixed with 10.0 ml of water, and shaken for 24 hrs. at room temperature.

5 Undissolved residue in each solution was removed by
filtration through 0.45 μ filter. The filtered solutions
were separated into two parts. One part of each solution
was injected in triplicate into chemiluminescent nitrogen
detector (model 8060, Antek Instruments) equipped with an
10 analytical loop of 5 μ l volume. The other part of each
filtered solution was assayed with total organic carbon
analyzer (model TOC-5000, Shimadzu Scientific Instruments,
Columbia, MD, USA). Results obtained by analysis of both
nitrogen content and carbon content of the saturated
15 solutions are presented in Figure 3. The results
presented in Fig.3 indicate that there is a good
correlation between the data obtained by the two assays.

The results of this example illustrate that the
assaying of an element content in the saturated solutions
20 of compounds allows one to measure the compound
concentrations, i.e. solubility of compounds, independent
of the particular element content measured.

Example 4. Measuring solubility of several different
compounds in buffer by the procedure according to the
25 present invention using two different assay protocols.

Allopurinol, bendroflumethiazide, butamben,
clofazimine, nitroflurazone, theophylline, nifedipine,
perphenazine, phenacetin, trimethoprim, and caffeine were
purchased from Sigma Chemical Company St. Louis, MO, USA)
30 and used without further purification. About 20 mg

5 quantities of each of these compounds were mixed with 10.0
ml of 0.15M NaCl in 0.01M universal buffer, pH 7.4
composed of appropriate quantities of acetic acid,
phosphoric acid, boric acid, and NaOH, and shaken for 24
hrs. at room temperature. Undissolved residue in each
10 solution was removed by filtration through 0.45 μ filter.
Parts of the filtered solutions were injected in
triplicate into chemiluminescent nitrogen detector (model
8060, Antek Instruments) equipped with an analytical loop
of 5 μ l volume. The other part of each filtered solution
15 was assayed with total organic carbon analyzer (model TOC-
5000, Shimadzu Scientific Instruments, Columbia, MD, USA).
Results obtained by analysis of both nitrogen content and
carbon content of the saturated solutions are presented in
Figure 4. The results presented in Fig.4 indicate that
20 there is a good linear correlation between the data
obtained by the two assays.

The results of this example illustrate that the
assaying of an element content in the saturated solutions
of compounds allows one to measure the compound
25 concentrations, i.e. solubility of compounds, independent
of the particular element content measured.

Example 5. Measuring solubility of different compounds
dissolved in DMSO by the procedure according to the
present invention.

5 Allopurinol, bendroflumethiazide, butamben,
clofazimine, nitroflurazone, theophylline, nifedipine,
perphenazine, phenacetin, sulfanilamide, and trimethoprim
were purchased from Sigma Chemical Company (St. Louis, MO,
USA) and used without further purification. Stock
10 solutions of each compound in DMSO with the concentration
of 5.0 mg/ml were prepared. The fixed volume of 450 μ l of
0.01M universal buffer containing 0.15M NaCl, pH 7.4 was
mixed with 9.2 μ l of stock DMSO solution of each compound.
The mixtures were shaken for 24 hrs. at room temperature.
15 Undissolved residue in each solution was removed by
filtration through 0.45 μ filter, and the solutions were
injected in triplicate into chemiluminescent nitrogen
detector (model 8060, Antek Instruments) equipped with an
analytical loop of 5 μ l volume.

20 The measured nitrogen content for each compound
solution was transformed into concentration units, and the
resulting experimental "DMSO solubility" values are
plotted in Figure 5 versus corresponding data obtained for
the same compounds in the same buffer using the procedure
25 described in Example 2. The data given in Fig. 5 indicate
clearly that there is a good correlation between the "DMSO
solubility" values obtained by the procedure and those
obtained with dry compounds obtained by the procedure
according to the present invention over the range from 1
30 μ g/ml up to ca.10 mg/ml. The correlation between the data
may be described by a linear relationship as:

5
$$S_{\text{exp}}^{\text{DMSO}} = -0.10 (\pm 0.15) + 1.524 (\pm 0.054) * S_{\text{exp}}$$

$$N = 11; r^2 = 0.9887; s = 0.4244,$$

where S_{exp} is the compound solubility measured experimentally by the procedure described; $S_{\text{exp}}^{\text{DMSO}}$ - solubility of the compound measured in the presence of DMSO; N is the number of compounds; r - correlation coefficient, s - standard error of estimate.

10

The above correlation indicates clearly that the solubility of compounds examined in the presence of DMSO generally exceeds that of the same compounds without DMSO.

15 However, the linear relationship observed can be used to re-calculate the measured solubility values from $S_{\text{exp}}^{\text{DMSO}}$ into S_{exp} values, or used for ranking the solubility of a studied series of compounds.

This example illustrates the possibility to use the procedures according to the present invention for compounds dissolved in an organic solvent, such as DMSO, for estimating the compound solubility in a given aqueous solution.

20

Example 6. Ranking solubility of different compounds by the procedure according to the present invention.

25

Allopurinol, bendroflumethiazide, butamben, clofazimine, nitroflurazone, theophylline, nifedipine, perphenazine, phenacetin, trimethoprim, and caffeine were purchased from Sigma Chemical Company (St. Louis, MO, USA)

5 and used without further purification. Stock solutions of
each compound in methanol with the concentration of 5.0
mg/ml were prepared. The fixed volume of 450 μ l of 0.01M
universal buffer containing 0.15M NaCl, pH 7.4 was mixed
with 9.2 μ l of stock methanol solution of each compound.
10 The mixtures were shaken for 24 hrs. at room temperature.
Undissolved residue in each solution was removed by
filtration through 0.45 μ filter, and the solutions were
injected in triplicate into chemiluminescent nitrogen
detector (model 8060, Antek Instruments) equipped with an
15 analytical loop of 5 μ l volume.

The measured nitrogen content for each compound
solution was transformed into concentration units, and the
resulting experimental "methanol solubility" values are
plotted in Figure 6 versus corresponding data obtained for
20 the same compounds in the same buffer using the procedure
described in Example 2. The data given in Fig. 6 indicate
clearly that there is a good correlation between the
"methanol solubility" values obtained by the procedure and
those obtained with dry compounds obtained by the
25 procedure according to the present invention over the
range from 1 μ g/ml up to ca.20 mg/ml. The correlation
between the data may be described by a linear relationship
as:

$$S_{\text{exp}}^{\text{MetOH}} = 0.055(\pm 0.047) + 1.015(\pm 0.008) * S_{\text{exp}}$$

30 $N = 10; r^2 = 0.9994; s = 0.1335,$

5 where S_{exp} is the compound solubility measured experimentally by the procedure described; $S_{\text{exp}}^{\text{MeOH}}$ - solubility of the compound measured in the presence of 2% methanol; N is the number of compounds; r - correlation coefficient, s - standard error of estimate.

10 The above correlation indicates clearly that the solubility of compounds examined in the presence of methanol generally exceeds that of the same compounds without methanol. However, the linear relationship observed can be used to re-calculate the measured
15 solubility values from $S_{\text{exp}}^{\text{MeOH}}$ into S_{exp} values, or can be used for ranking the solubility of a studied series of compounds.

This example illustrates the possibility to use the procedures according to the present invention for
20 compounds dissolved in an organic solvent, such as methanol, for estimating the compound solubility in a given aqueous solution.

The results illustrated by the above examples demonstrate that the procedures according to the present
25 invention facilitate high-throughput measurements of solubility for a wide variety of compounds.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into

5 this application in order to more fully describe the state
of the art to which this invention pertains.

It will be apparent to those skilled in the art that
various modifications and variations can be made in the
present invention without departing from the scope or
10 spirit of the invention. Other embodiments of the
invention will be apparent to those skilled in the art
from consideration of the specification and practice of
the invention disclosed herein. It is intended that the
specification and examples be considered as exemplary
15 only, with a true scope and spirit of the invention being
indicated by the following claims.

Claims:

1. A method for determining the solubility of a compound in a selected solvent, comprising the steps of:
 - a) preparing a mixture of a quantity of the compound with a volume of the selected solvent;
 - b) incubating the mixture of compound and solvent, whereby compound can dissolve in the solvent to form a solution of the compound;
 - c) removing undissolved compound from the mixture, thereby providing a quantity of the solution of the compound;
 - d) determining the amount of a selected constituent of the solution of the compound resulting from(c), wherein the determination does not include a comparison of physical properties of one or more solutions containing known concentrations of the compound and physical properties of the solution of the compound; and
 - e) calculating the solubility of the compound in the selected solvent by determining the amount of the compound present in a determined quantity of the solution of the compound.
2. The method of claim 1, wherein the quantity of the compound present in (a) is in excess of the amount of the compound that can be dissolved in the selected solvent.

3. The method of claim 1, wherein the amount of the selected solvent present in (a) is insufficient to dissolve the amount of the compound.
4. The method of claim 1, wherein the step of incubating the mixture of the compound and the solvent is continued until the amount of compound dissolved in the solvent approaches a value equal to a specified percentage of the equilibrium solubility value.
5. The method of claim 4, wherein the specified percentage of the equilibrium solubility value is selected from the group of values consisting of 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 75%, 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98%, and 99%.
6. The method of claim 1, wherein the step of incubating the mixture of the compound and the solvent is continued until thermodynamically equilibrated phase separation occurs, wherein a phase includes compound dissolved in solvent and at least one other phase.
7. The method of claim 6, wherein the amount of the compound present in the phase including compound dissolved in the solvent equals an amount equal to a specified percentage of the equilibrium solubility value.
8. The method of claim 7, wherein the specified percentage of the equilibrium solubility value is selected from the group of values consisting of 5%, 10%, 15%, 20%, 25%, 30%, 35%,

40%, 45%, 50%, 75%, 80%, 85%, 90%, 93%, 95%, 96%, 97%, 98%, and 99%.

9. The method of claim 1, wherein determining the amount of a selected constituent of the solution of the compound resulting from(c) consists of determining the amount of a specified element present in the compound.
10. The method of claim 9, wherein determining the amount of the specified element present in the compound is accomplished by use of a detector selected from the group consisting of a universal equimolar chemiluminescent nitrogen detector, equimolar chemiluminescent sulfur detector, and a total organic carbon detector.
11. The method of claim 1, wherein the method comprises measuring the overall content of a given chemical element present in the compound dissolved in a selected volume of the solution.
12. The method of claim 11, wherein the method comprises measuring the overall content of a given chemical element present in the compound and in the solvent in a selected volume of the solution.
13. The method of claim 11, wherein the method comprises measuring the overall content of more than one given chemical element present in the solution of the compound.
14. The method of claim 13, wherein at least one chemical element is present in the compound dissolved in the

solution of the compound and at least one chemical element is present in the selected solvent.

15. The method of claim 1, wherein the amount of at least one chemical element is determined in a specified volume of solution and wherein the volume is analytically determined.
16. The method of claim 15, wherein the volume is analytically determined using volumetric measurements.
17. The method of claim 15, wherein the volume is determined by determination of the amount of an element present in the solution.
18. The method of claim 1, wherein determining the solubility of the compound in the selected solvent comprises converting the overall content of a selected chemical element in solution into the concentration of the selected compound from the molar content of the element in the compound.
19. The method of claim 18, wherein determining the solubility of the compound in the selected solvent comprises use of a detector-specific universal quantitative relationship between the chemical element content and quantity of the element-containing compound in a determined volume of solution of the compound.
20. The method of claim 19, wherein the determined volume of solution of the compound is determined by volumetric measurement.

21. The method of claim 19, wherein the determined volume of solution of the compound is determined by determining the quantity of a specified component of the solution.
22. The method of claim 21, wherein the specified component of the solution is an element present in the solvent.
23. The method of claim 21, wherein the specified component of the solution is a component present in both the solvent and the compound.
24. The method of claim 1, wherein the selected solvent is an aqueous solvent.
25. The method of claim 1, wherein the selected solvent is a nonaqueous solvent.
26. The method of claim 25, wherein the selected solvent is a mixture of a nonaqueous and an aqueous solvent.
27. The method of claim 26, wherein the selected solvent comprises a percentage of DMSO.
28. The method of claim 27, wherein the percentage of DMSO is selected from the group consisting of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.4, 2.8, 3.2, 3.6, 4.0, 4.5, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90 or 100%.
29. The method of claim 1, wherein the compound contains a known quantity of a specified element per unit of mass.

30. The method of claim 29, wherein the specified element is selected from the group consisting of sulfur, carbon, nitrogen, hydrogen, phosphorus and oxygen.
31. The method of claim 1, wherein a) includes contacting the quantity of the compound with a volume of a first solvent to form a first mixture and contacting a quantity of the first mixture with a volume of a further solvent.
32. The method of claim 31, wherein the first solvent comprises an organic solvent.
33. The method of claim 32, wherein the organic solvent is DMSO.
34. The method of claim 31, wherein the compound is dissolved in the first mixture.
35. The method of claim 34, wherein the compound dissolved in the first mixture comes out of solution when contacted with further solvent.
36. The method of claim 31, wherein at least a portion of the first solvent is removed.
37. The method of claim 36, wherein the first solvent is removed by evaporation or by application of vacuum.
38. A method for determining the solubility of chemical compounds, including organic, inorganic and organo-metallic compounds, comprising the steps of:

- a) preparing a mixture of unknown excess amount of compound sample with a fixed volume of a solvent of choice;
- b) maintaining a sample of the aforementioned mixture until thermodynamically equilibrated phase separation occurs;
- c) withdrawing aliquots of the saturated solution from the thermodynamically equilibrated mixture;
- d) analyzing the concentration of the compound in the solution resulting from step (c) by measuring the overall content of a given chemical element in a fixed volume of the solution; and
- e) converting the overall content of a given chemical element in the solution into the concentration of the tested compound from the molar content of the chemical element in the compound, and the detector-specific universal quantitative relationship between the element content and quantity of the element-containing compound in a fixed solution volume.

39. The method of claim 38, wherein the solvent for the preparation of mixture is of aqueous or organic nature and a known quantity of the compound contains a known amount of nitrogen or sulfur.

40. The method of claim 38, wherein the selected solvent comprises salt and/or buffer salt additives.
41. The method of claim 40, wherein the pH of the selected solvent has a pH within one-half a pH unit of a pH value selected from the group consisting of 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 13.0, and 14.0.
42. The method of claim 1, wherein the compound is a dry compound.
43. The method of claim 42, wherein the total weight of the dry compound does not exceed 1 gram.
44. The method of claim 42, wherein the fixed volume of the solvent of choice does not exceed 5.0 mL in the mixture.
45. The method of Claim 38, wherein the maintaining a sample of the aforementioned mixture until thermodynamically equilibrated phase separation occurs is performed under shaking, mixing, vortexing, ultrasound treatment, temperature treatment, or any other external treatment for facilitating the process.
46. The method of Claim 38, wherein the removing the undissolved residue from the thermodynamically equilibrated mixture is performed by centrifugation, filtration, or other procedure.
47. The method of Claim 38, wherein the overall content of a given chemical element, such as carbon, sulfur, or

nitrogen, is measured in a fixed volume of a compound solution.

48. The method of Claim 47, wherein the overall content of a given chemical element in a fixed volume of a compound solution is transformed into concentration of the compound.
49. A method for determining the solubility of one or more compounds in one or more selected solvents, comprising preparing two or more mixtures of compound and solvent and determining the solubility of each according to any of claims 1-48.
50. The method of claim 49, wherein the two or more mixtures are contained in a multi-well container.
51. The method of 50, wherein the multi-well container is a microtiter plate.
52. The method of claim 51, wherein the multi-well container has at least 4 wells.
53. The method of claim 52, wherein the multi-well container has at least 8 wells.
54. The method of claim 53, wherein the multi-well container has at least 16 wells.
55. The method of claim 54, wherein the multi-well container has at least 32 wells.

56. The method of claim 55, wherein the multi-well container has at least 48 wells.
57. The method of claim 56, wherein the multi-well container has at least 64 wells.
58. The method of claim 57, wherein the multi-well container has at least 80 wells.
59. The method of claim 58, wherein the multi-well container has at least 96 wells.
60. An apparatus for determining the solubility of a compound in a selected solvent, comprising:
 - a) a mixing device that combines a quantity of a compound with a volume of a selected solvent, thereby forming a mixture in a container;
 - b) an incubating device that maintains the mixture at determined conditions for, optionally, a determined period of time;
 - c) a separating device that removes undissolved compound from the mixture, thereby providing a quantity of a solution of the compound; and
 - d) a detector that detects the amount of a selected constituent in the solution of the compound.

Figure 1

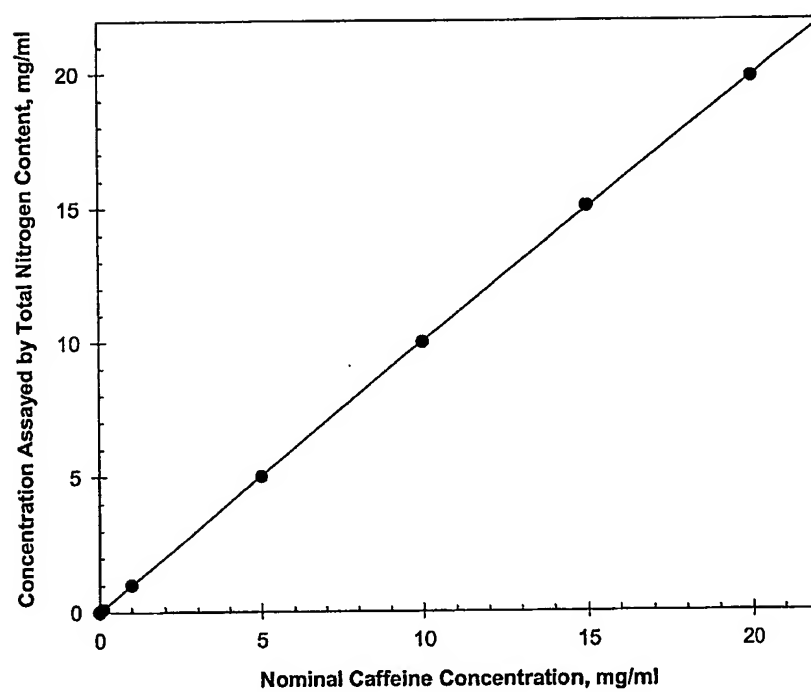


Figure 2

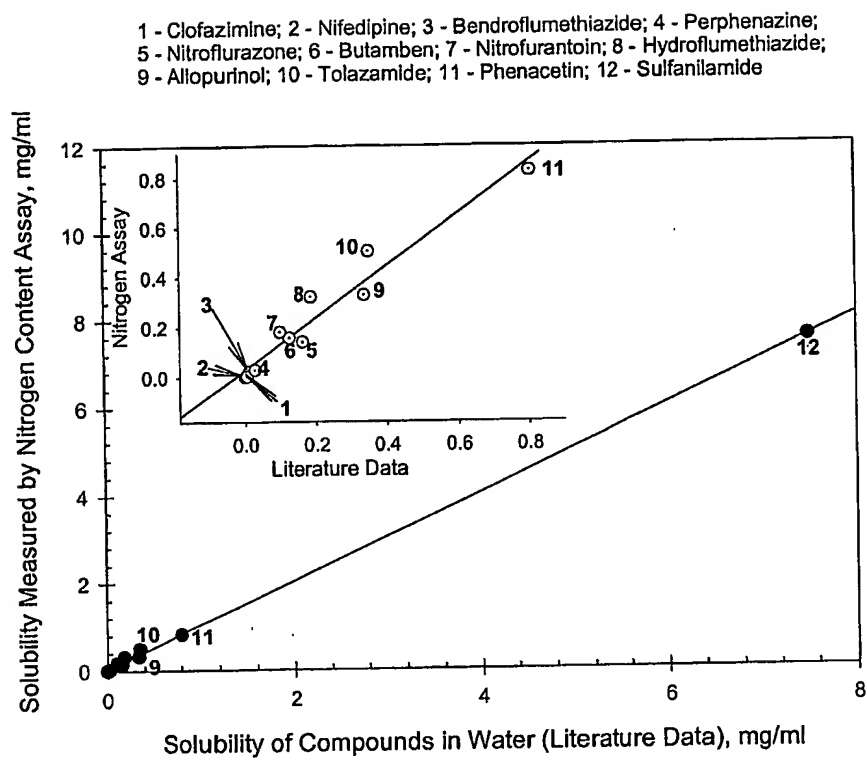


Figure 3

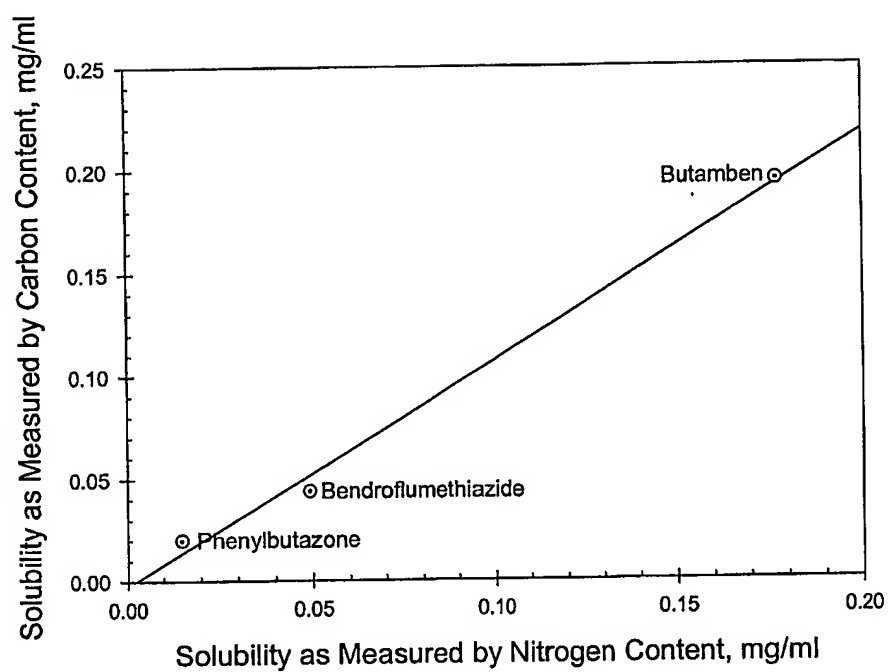


Figure 4

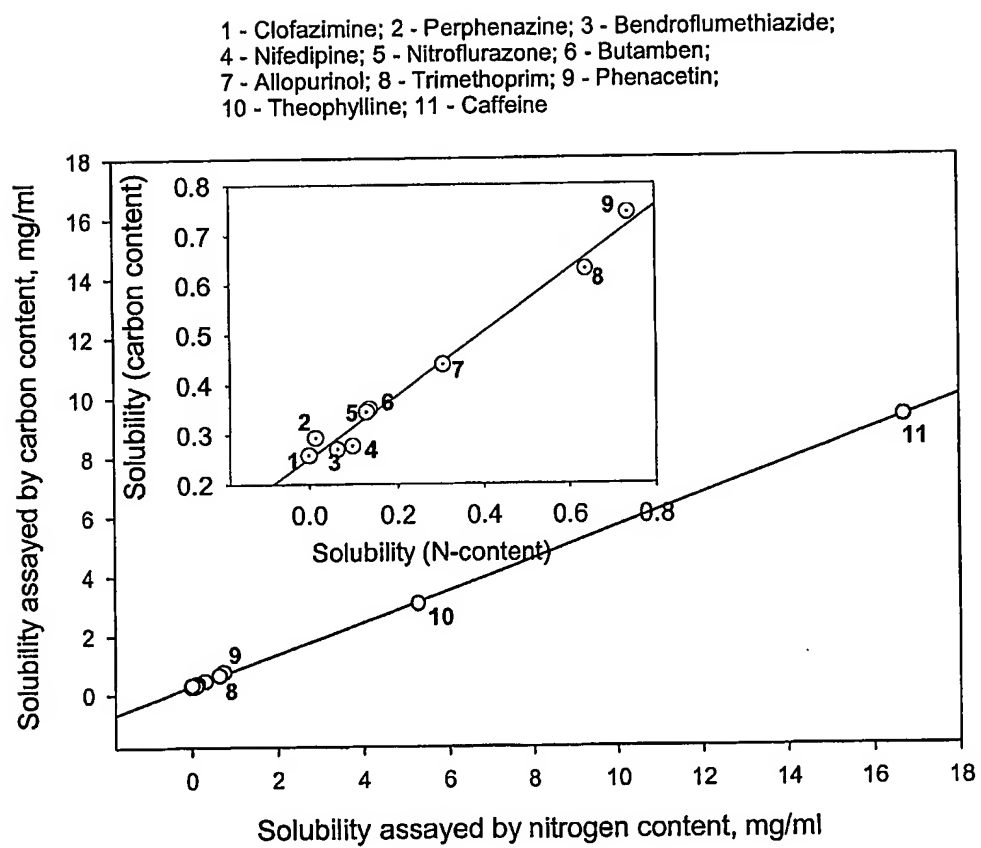


Figure 5

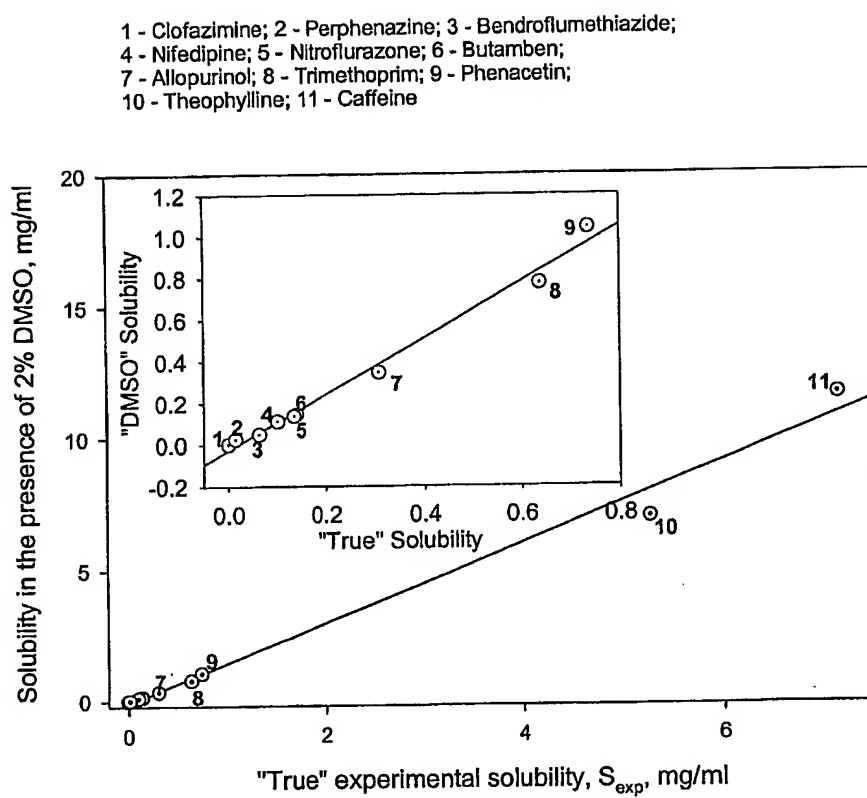
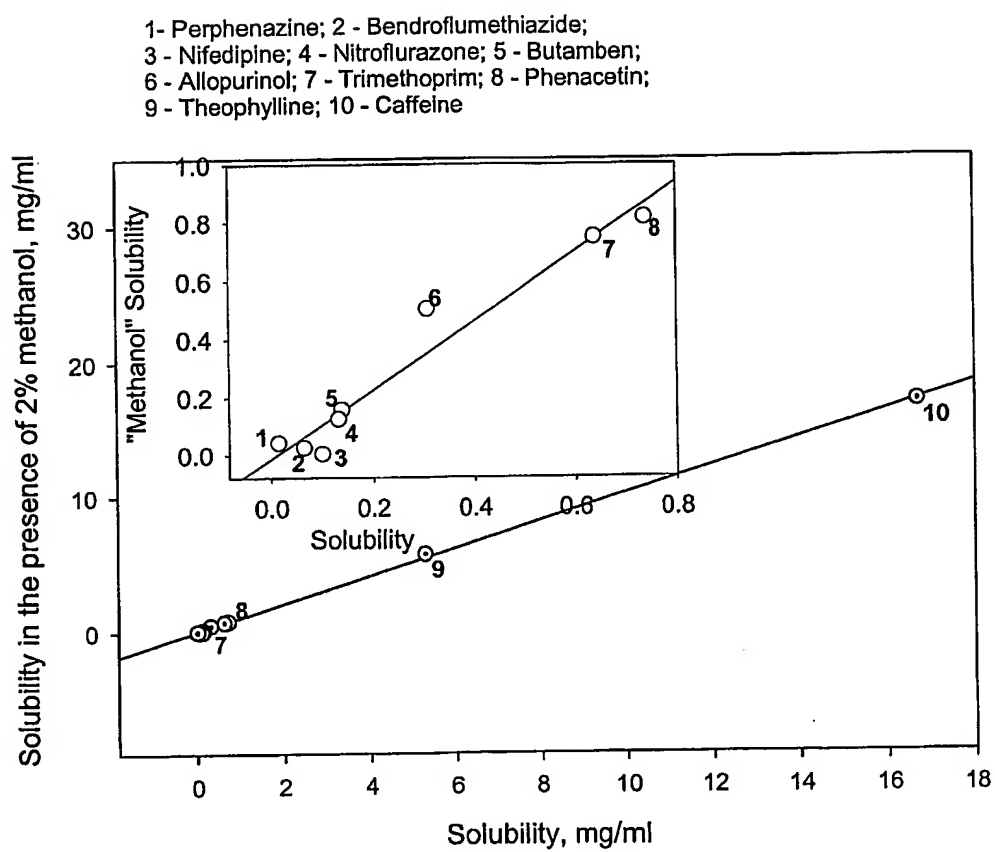


Figure 6



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/26019

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : G01N 21/76

US CL : 436/106, 114, 119, 123, 146, 172; 422/52, 78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/106, 114, 119, 123, 146, 172; 422/52, 78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Chemical Abstract No. 105:60254. CAS Online, Columbus, Ohio. TAKANO et al. Solubility measurement of liquid organic compounds in water. Nippon Kagaku Kaishi, 1985, Vol. 11, pages 2116-2119. See entire abstract.	1-24, 29-30, 38, 45-49, 60
X	BEVAN et al. A high-throughput screening method for the determination of aqueous drug solubility using laser nephelometry in microtiter plates. Analytical Chemistry, April 15, 2000, Vol. 72, No. 8, pages 1781-1787, especially page 1781, column 2, and page 1787, column 2.	1-8, 24-28, 31-37, 42-44, 49-60
A	US 5,008,204 A (STEHLLING) 16 April 1991 (16.04.1991), see column 2, lines 37-68 and column 3, lines 19-21.	1-60

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

18 September 2002 (18.09.2002)

Date of mailing of the international search report

03 OCT 2002

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks

Box PCT

Washington, D.C. 20231

Facsimile No. (703)305-3230

Authorized officer

Jan M. Ludlow

Telephone No. (703) 308-0661

INTERNATIONAL SEARCH REPORT

PCT/US02/26019

Continuation of B. FIELDS SEARCHED Item 3:

EAST, CAS Online. search terms: measur? or detect? or determin? or test?; solubili?; toc or "total organic carbon"; chemilumines?; nitrogen or sulfur or sulphur